Journal of Forensic and Legal Medicine 19 (2012) 77–82

Contents lists available at SciVerse ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Original communication

Simultaneous analysis of some club drugs in whole blood using solid phase extraction and gas chromatography–mass spectrometry

André L. Castro M.Sc. Forensic Expert [a,b,*], Sónia Tarelho M.Sc. Forensic Expert [a,b], Armando Silvestre PhD Professor [c], Helena M. Teixeira M.Sc., PhD Professor, Director [a,b,d,e]

[a] National Institute of Legal Medicine, Forensic Toxicology, Jardim Carrilho Videira, 4050-067 Porto, Portugal
[b] CENCIFOR – Forensic Sciences Centre, Portugal
[c] CICECO and Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810 Aveiro, Portugal
[d] Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[e] Faculty of Medicine, University of Porto, Porto, Portugal

ARTICLE INFO

Article history:
Received 12 July 2011
Received in revised form
6 October 2011
Accepted 12 December 2011
Available online 4 January 2012

Keywords:
Club drugs
GC–MS
SPE
Whole blood

ABSTRACT

The use of psychoactive substances to improve social relations and increase body energy, in *Rave Culture*, has raised many legal and health public concerns, both for illicit trade and consumption. Therefore, forensic toxicology plays an important role in this area, mainly linked to the detection and quantitation of these substances, both *in vivo* and in *post-mortem* samples. In fact, at the moment, forensic sciences have been under public authorities' scrutiny and critical look, due to the increasing attention of the media and public opinion, always applying for the use of scientific knowledge to help solving forensic cases. However, forensic toxicology results are only reliable to solve legal cases if all the analytical methodologies used are appropriately validated.

In this work, a methodology for the extraction and analysis of 7-aminoflunitrazepam, buprenorphine, flunitrazepam, ketamine, methadone, phencyclidine (PCP) and D-propoxyphene was developed for whole blood samples, with solid phase extraction (SPE), using OASIS® MCX SPE columns, and gas chromatography coupled to mass spectrometry.

The procedure presented here proved to be reliable, specific, selective and sensitive, with good LODs and LOQs and good precision.The adoption of a SPE procedure with an automatic SPE extraction device, allowed an increased level of automation in sample treatment, being contemporarily less time-consuming, increasing productiveness, and allowing good recovery and appropriate selectivity being, also, simple and reproducible. The simultaneous detection and quantitation of all compounds by the same extraction and detection methodology is crucial and has a great potential for forensic toxicology and clinical analysis.

1. Introduction

Although ethanol remains as the most used substance for socialising purposes, nowadays it has been simultaneously used with some psychoactive drugs in order to intensify social and sensorial experiences.[1–3] Through the 1990s, some new compounds, associated to the *rave subculture*, have been adopted, on an always growing rate.[1–7] Club drugs include, among others, flunitrazepam, γ-hydroxibutirate (GHB), ketamine and 3,4-methile nedeoximetanfetamine (MDMA), also known as *Ecstasy*.[1,3,4,8–12]

This group includes a wide range of substances, from stimulants to depressants, to hallucinogens, among others, and it is fair to consider that some of the compounds (GHB, ketamine, phencyclidine, methadone and D-propoxyphene) are not really new, but are abused with totally different aims from those that arose their synthesis.[4,5,13,14] The use of these compounds has become increasingly common among people, leaving the club scene and entering in the mainstream of night diversion and in starting school individuals, alone or in a polydrug use scheme, leading the authorities to new fears and to the need of new control developments and monitoring programs.[4,7,9,11,15,16]

The price and the easy disposal as pills, powders, liquids or others, guarantee great popularity to these compounds, enhancing all the illicit dealing associated.[1,4] The combined use of some of the substances, known as *polydrug use*, has also become the most

* Corresponding author. National Institute of Legal Medicine, Forensic Toxicology, Jardim Carrilho Videira, 4050-067 Porto, Portugal. Fax: +351 222018069.
E-mail address: andre.castro@dpinml.mj.pt (A.L. Castro).

1752-928X/$ – see front matter © 2011 Elsevier Ltd and Faculty of Forensic and Legal Medicine. All rights reserved.
doi:10.1016/j.jflm.2011.12.006

noticed behaviour between users, whether they are *ravers* or just curious consumers.[11]

Substances' detection in *post-mortem* samples raises different concerns, considering not only the sampling procedures, but also the sample treatment and storage and, not less important, the results interpretation. Forensic toxicology brings always real-time challenges, both in analytical terms, as well as in the results evaluation and interpretation. Thus, toxicological data should never be used by itself, but always considering all the case circumstantial information, as crime scene data, autopsy data and, if possible, social, individual and historical information.[17–20]

Procedures and methodologies development, both for systematic screening or for compounds confirmation/quantitation are crucial to understand any substance influence, in the cause of death or clinical influence in the examined person.[21]

A GC–MS methodology was fully developed and validated for the detection and quantitation of 7-aminoflunitrazepam, buprenorphine, flunitrazepam, ketamine, methadone, phencyclidine (PCP) and D-propoxyphene, with phencyclidine-d_5 (PCP-d_5) as internal standard (IS), in whole blood samples.

2. Materials and methods

2.1. Materials, standards and chemicals

All pure standards of the studied substances (and of the IS) were purchased from Lipomed AG (Arlesheim, Switzerland). Each standard compound was dissolved in methanol and stored at −20 °C, at different concentrations: PCP, flunitrazepam and 7-aminoflu nitrazepam (1 mg/L); PCP-d_5 (5 mg/L); D-propoxyphene, methadone and buprenorphine (10 mg/L) and ketamine (100 mg/L).

All solvents (methanol, acetonitrile, ethyl acetate, chloridric acid Titrisol® and amonia 25%) were of analytical or gradient grade and were purchased from E. Merck (Darmstadt, Germany). BSTFA–TM CS 99:1 was purchased from Supelco (Saint Louis, USA). Water was purified by a Milli-Q system obtained from Millipore (Molsheim, France).

Solid phase extraction (SPE) procedure was performed in an automatic SPE Equipment, Aspec XL® 271 (Gilson™), with Oasis® MCX (3 cc; 60 mg) solid phase extraction columns, obtained from Waters™ (WATERS Corporation, USA).

2.2. Sample preparation and SPE extraction

Control and calibration samples were prepared by spiking drug-free samples with standard solutions.

One millilitre of drug-free whole blood samples was diluted in 4 mL of deionised water and spiked with all the studied drugs at different concentrations ranges (Table 1). Deuterated internal standard (IS), PCP-d_5, was used by adding 50 μL of a 1 mg/L solution to all the samples examined (final concentration: 250 ng/mL). The tubes were vortexed for 30 s, allowed to sit for 1 min and centrifuged for 30 min at 4000 rpm.

A solid phase extraction technique was carried out to whole blood samples, using OASIS® MCX (Waters™) with mixed-mode behaviour, considered both for the retention of basic, neutral and/or acid compounds.[22,23] SPE columns were conditioned by sequentially adding 2 mL methanol and 2 mL water. The prepared samples were poured onto the conditioned columns and allowed to drain at a 1–2 mL/minute flow. Each column was then washed by the sequential addition and elution of 2 mL water, 2 mL hidrochloric acid 0.1 M and 2 mL methanol 5% in water, and dried under air pressure for 1 min. Elution was performed by adding 2 mL acetonitrile/methanol (70:30, v/v) (to collect the acid and neutral compounds) followed by the addition of 2 mL ethyl acetate/NH_4^+ (95:5, v/v) (to collect the basic compounds).

After solvent evaporation, the residues were redissolved with 200 μL of methanol and vortexed, to increase recovery from tube walls. The extracts were transferred to vial reaction inserts, evaporated and derivatised with 50 μL of BSTFA+TMCS 99:1, 60–70 °C for 30 min. One microlitre of the final solution was then injected into the GC–MS system.

2.3. GC–MS analysis

GC–MS analysis was conducted on an Agilent 6890 series, with a liquid autosampler Agilent 7683 series and an Agilent 5973N Series mass selective detector with a Factor Four VF 17-MS (0.25 mm/15 m/0.25 μm) column from Varian.

The injector and detector temperatures were set at 300 °C and 280 °C, respectively. The column temperature was initially held at 70 °C for 3 min, increased to 290 °C (40 °C/min), and held at 290 °C for 6 min. A mass selective detector in SIM mode coupled to GC was used both for qualitative and for quantitative analysis, with a solvent delay of 2.50 min.

Quantitation was achieved by the addition of one deuterated analogue as internal standard, PCP-d_5. The compounds were quantified by selected ion monitoring (SIM) of *m/z* 208 (for D-propoxyphene), 200 (for PCP), 180 (for Ketamine); 294 (for Methadone), 286 (for Flunitrazepam), 283 (for 7-aminoflunitrazepam), 450 (for Buprenorphine), and 205 for the deuterated internal standard, PCP-d_5. Nevertheless, at least three ions were monitored for all the studied substances identification (Table 2).

2.4. Validation studies

To evaluate peak-purity and selectivity, 10 different blank samples (no analyte or internal standard added) were analysed to check for peaks that might interfere with detection of the analyte or internal standard (IS). Negative samples (blank samples + IS) were also analysed, to verify the absence of native analyte in the IS solution.

Calibration was performed by spiking whole blood samples with the studied substances at different concentrations ranges (Table 1).

Table 1
LOD, LOQ and linearity data.

Compound	Concentration range (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	r^2	s_1^2	s_2^2	F_{calc}	$F_{crit\ (95\%)}$
PCP	15–100	4.73	14.32	0.993	5.284	18.99	3.595	4.026
Ketamine	250–3000	274.01	830.35	0.994	16602.068	17809.94	3.408	
Methadone	100–1500	173.64	526.17	0.992	6577.071	39816.59	2.707	
D-propoxifene	250–2000	189.21	573.38	0.992	12830.469	56585.12	3.103	
Flunitrazepam	15.3–102	7.30	22.14	0.994	2333.134	3254.12	1.394	
7-Aminoflunitrazepam	16.5–110	6.80	20.61	0.998	97840.679	210716.65	2.154	
Buprenorphine	10–500	47.97	145.38	0.995	79.664	256.42	3.218	

r^2: Correlation factor; s_1^2: variance obtained in the lower limit of the calibration curve; s_2^2: variance obtained in the upper limit of the calibration curve; F_{calc}: calculated *F* value; and $F_{crit\ (95\%)}$: critical *F* value, obtained from Snedecor/Fischer's *F* distribution table.

Table 2
GC–MS parameters established for PCP, D-propoxyphene, methadone, flunitrazepam, 7-aminoflunitrazepam, buprenorphine, ketamine and internal standard (PCP-d5).

Compound	RT (min)	*m/z**	*m/z***
PCP	7.05	200	91, 242, 243
PCP-d5	7.05	205	–
Ketamine	7.21	180	180, 182, 209
D-propoxyphene (includes thermal degradation compounds)	7.76	208	58, 91, 115, 193
Methadone	7.77	294	72, 294, 309
Flunitrazepam	9.78	286	238, 266, 285, 286, 312, 313
7-Aminoflunitrazepam	10.36	283	254, 255, 282, 283
Buprenorphine	12.54	450	450, 451, 482, 506

* *m/z*: Selected ion for quantitation.
** *m/z*: Selected ions for confirmative identification.

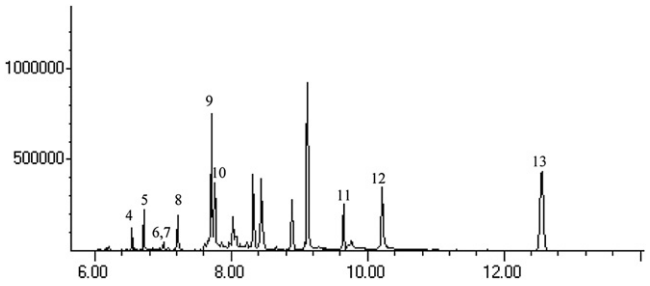

Fig. 1. Compounds mix chromatogram: D-propoxyphene (4, 5, 10); PCP and PCP-d_5 (6, 7); ketamine (8); methadone (9); flunitrazepam (11); 7-aminoflunitrazepam (12); and buprenorphine (13).

Five calibrators were used to generate the standard curve, each calibrator injected in triplicate.

The limit of detection (LOD) and quantitation (LOQ) were estimated from extracted samples spiked with the studied compounds. The limits of detection (LOD) and quantitation (LOQ) were established using the residual standard deviation ($S_{y/x}$) and the slope of the linear regression (b), as LOD = 3.3 $S_{y/x}/b$ and LOQ = 10 $S_{y/x}/b$.[24,25]

The recovery of SPE was determined by repeated analysis of five samples spiked at three different levels of the studied substances (low, medium and high level), and with the internal standard spiked at a final 250 ng/mL concentration. The extraction recovery was determined by comparing the representative peak areas of extracted drug-free samples spiked before extraction with the peak area of drug-free samples fortified after the extraction at the same concentration levels. The samples were injected in the equipment and the results were compared in terms of percentage. Thus, recovery was calculated by using the formula: (Compound Peak Area pre-SPE/IS Peak Area)/(Compound Peak Area pos-SPE/IS Peak Area) × 100, and represented as percentage (%).

The precision was studied evaluating intra-day coefficient of variation values. These were determined by replicate analyses ($n = 10$) of blood aliquots. Three concentration levels were selected for validation, as shown in Table 3.

2.5. Real cases application

Samples were collected at the Forensic Pathology Department (FPD) or at the Medico-Legal Department (MLD) of the North Branch of the National Institute of Legal Medicine (INML, I.P.), under the routine sample collection protocol.

The procedure was applied according to the developed method, using contemporaneous positive and negative controls, prepared with blank whole blood samples.

3. Results and discussion

All the compounds were detected using the selected ions and according to the specific retention time (RT) (Fig. 1).

The D-propoxyphene detection has shown a predicted resistance: the detection of three peaks at different retention times, 6.20, 6.54 and 7.76 (Fig. 2). As described previously by other authors, the first two peaks represent D-propoxyphene thermal degradation products and the third peak corresponds to its main compound.[26] In fact, this substance suffers thermal degradation in the GC–MS injector, with the formation of two other products, due to the propionic acid loss. However, during the study, we observed that at different injector temperatures (between 200 °C and 300 °C) the compound degradation behaviour was not reproducible, but all the fortified samples had the main compound peak and the two thermal degradation compounds peaks. Thus, it was possible to

Table 3
Between-run precision and recovery results.

Compound (ng/mL)	Spiked value	Obtained value	σ	CV (%)	[Compound]	Recovery (%)
PCP	15	17.5	0.62	4	15	105
	50	68.5	1.86	3	50	108
	100	128	4.4	3	100	84
Methadone	100	158	21.6	14	100	73
	500	635	56.2	9	500	62
	1500	1741	93.2	5	1500	66
D-propoyphene	250	262	25.5	10	250	177
	1000	1142	73.6	6	1000	91
	2000	1728	69.0	5	2000	91
Ketamine	250	300	2.9	1	250	125
	1000	609	33.1	5	1000	86
	3000	1746	155.9	9	3000	86
Flunitrazepam	10.2	12.2	0.75	6	15.3	57
	51	45	3.6	8	51	91
	100	123	7.1	6	102	94
7-Aminoflunitrazepam	16.5	27.0	5.33	14	16	64
	55	37.1	5.76	15	55	95
	110	159	17.4	11	110	90
Buprenorphine	25	15.9	0.02	0.1	25	65
	100	90.7	9.40	10	100	114
	500	348	35.2	10	500	97

σ: Standard deviation; and CV: coefficient of variation.

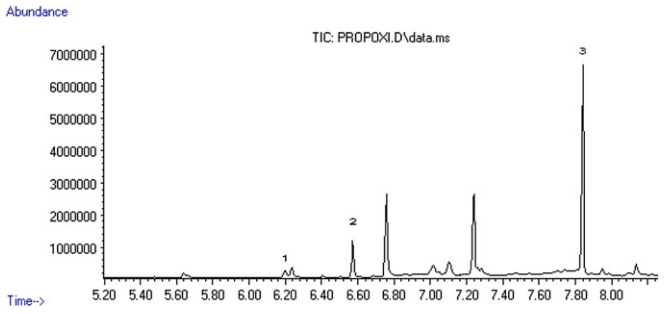

Fig. 2. Detection of the three peaks for D-propoxyphene: 1 + 2: D-propoxyphene thermal degradation products; 3: D-propoxyphene main compound.

conclude that the sample was positive for D-propoxyphene. We also noticed that the obtained mass spectra were reproducible, indicating that a positive result could be distinguished from a negative result (Fig. 3).

All 10 samples were free of co-eluting peaks at the retention times of the corresponding studied substances and the corresponding IS. The method showed that there was no interference from the sample matrix (absence of any interfering peak) and proved, thus, to be selective and specific for 7-aminoflunitrazepam, buprenorphine, flunitrazepam, ketamine, methadone, phencyclidine (PCP) and D-propoxyphene.

Calibration curves were constructed in order to include, not only lethal or toxic values but also therapeutic levels, in order to increase the procedure usefulness (Table 4).[17,27] Thus, calibration was performed by spiking whole blood samples with the studied substances at different concentrations ranges (Table 1). Five calibrators were used to generate the standard curve, each calibrator injected in triplicate. The studied curves have shown good linearity for each particular range for all the considered compounds, with good correlation coeficients, $r^2 > 0.99$ (Table 1).

However, once again, D-propoxyphene has shown some particularities. Due to the thermal degradation mentioned before, it was not possible to obtain reliable quantitation values or even calibration curves using only the main compound analytical signals. In order to avoid this problem, the peak areas of the ion 208 obtained for all the three compounds (main compound and degradation products) were studied (Fig. 3) and manually integrated. The three different peak areas ($A_1 + A_2 + A_3$) were summed and used as one single value for quantitation purposes, as shown in equation: $(A_1 + A_2 + A_3)/A_{IS} = m \times [\text{D-propoxiphene}]/[IS] + b$, being m the calibration curve slope and b the intercept. The obtained values accomplished all the parameters of linearity for the defined concentration range, being, then, accepted as reliable. On the other hand, all the obtained validation parameters were in an acceptable range using this approach.

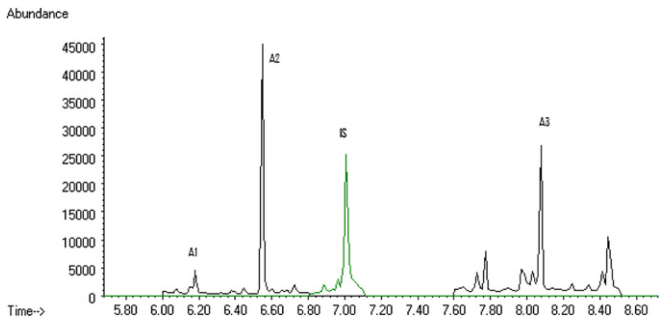

Fig. 3. Quantitation ion of the three peaks for D-propoxyphene: A1 + A2: D-propoxyphene thermal degradation products (ion 208); A3: D-propoxyphene main compound (ion 208); IS: PCP-d_5 as Internal Standard (ion 205).

The extreme points behaviour in each calibration curve were also evaluated, using a variance homogeneity test (F test). Ten aliquots for each compound were prepared in two concentrations, corresponding to the first (minimum) and the last (maximum) level of the calibration curve (Table 1). The variance (s_1^2 and s_2^2) values obtained were used to calculate the F value, which was, then, compared to a critical F value, in Snedecor/Fischer's F distribution table, considering a 95% confidence degree (F_{crit}). The calculated value was obtained by the following equation: $F_{calc} = s_2^2/s_1^2$. If the calculated value was smaller than the critical F value, the variance differences were not statistically significant, approving the curve range.

The limits of detection (LOD) and of quantitation (LOQ) achieved for each compound are described in Table 1. The studied procedure proved to have good limits both for therapeutic and toxic values, except for flunitrazepam and buprenorphine, since the LOQ for flunitrazepam was higher than the corresponding lower therapeutic levels and buprenorphine has shown LOD and LOQ also higher than the therapeutic levels. Nevertheless, the good results obtained in proficiency tests with blind samples including buprenorphine at a range starting at 1 ng/mL levels, with $|Z\text{-score}| < 2$ in all tests, have shown that the method proved to have good results for buprenorphine, starting at therapeutic levels, allowing an extra security when using this method in samples with therapeutic levels.

Very low LOD and LOQ concentrations (around sub-therapeutic levels) were achieved for ketamine, allowing important interpretation data whenever drug-facilitated sex abuse cases are concerned, since simple detection or small concentration values may be confirmed.

Data on between-run precision is presented in Table 3. As suggested by Christian and Castro,[24,25] precision levels were predicted considering all the studied work ranges, overlapping the fact that some values could be lower than the determined LODs. The calculation formulas used for LOD and LOQ considered the original work range and represented higher values than the first level of the calibration curve. Nonetheless, good accuracy and precision were obtained for all the compounds, with CV values clearly below 15% at the three studied concentration levels. However, the real samples analysis was performed with contemporary calibration curves starting with higher LOQ values.

The obtained recoveries have varied between compounds, as follows: PCP (102%), ketamine (81%), Methadone (68%), D-propoxyphene (77%), flunitrazepam (84%), 7-aminoflunitrazepam (79%) and buprenorphine (87%) (Table 3). Until now, no study has been published with all these substances included in the same method, when considering SPE procedures and GC–MS single quadrupole detection, and the existent ones only refer to the substances developed individually.[28] Moreover, the results now achieved are similar or better than the existent ones, mainly considering the recovery data.[28] Nevertheless, some of the referred techniques in the review are completely different, as to extraction procedures is concerned, it considers liquid–liquid extraction, solid

Table 4
Therapeutic and toxic ranges for PCP, D-propoxyphene, methadone, flunitrazepam, 7-aminoflunitrazepam, buprenorphine and ketamine.

Compound	Therapeutic range	Toxic range
PCP	7–24 ng/mL	>25 ng/mL
Ketamine	0.5–6.5 mg/L	7 mg/L
D-propoxyphene	50–750 ng/mL	>1000 ng/mL
Methadone	50–750 ng/mL	1000 ng/mL
Flunitrazepam	5–15 ng/mL	50 ng/mL
7-Aminoflunitrazepam	n/d	n/d
Buprenorphine	1–10 ng/mL	>200 ng/mL

n/d: Not defined.

Table 5
Results of the analysed real cases.

Sex	Age	Samples source	Ethiology	Detected club drugs	Other detected substances
F	ND	IV	Sexual abuse	N	N
F	16	IV	Sexual abuse	N	N
M	ND	PM	Drug addiction	N	N
M	ND	PM	Drug addiction	Methadone: 1435 ng/mL	Morphine: 270 ng/mL, Benzoylecgonine: 770 ng/mL, Oxazepam: 1.9 mg/L
M	19	PM	Domestic accident	N	N
F	ND	IV	Sexual abuse	N	N
F	ND	PM	Domestic accident	Ketamine: 3.8 mg/L	Midazolam: 0.13 mg/L, Lidocaine: 0.29 mg/L, Tramadol: 7.2 mg/L
M	35	PM	Domestic accident	Methadone: 647 ng/mL	Diazepam: <0.14 mg/L, Oxazepam: <0.13 mg/L, Nordiazepam: 0.55 mg/L
M	24	PM	Drug addiction	Methadone: 371 ng/mL	EtOH: 0.2 g/L; Alprazolam: 0.14 mg/L
M	35	PM	Road accident	Ketamine: 5.3 mg/L	EtOH: 1.04 g/L; Morphine: <25 ng/mL; Codeine: <25 ng/mL
M	45	PM	Drug addiction	Methadone: 533 ng/mL	EtOH: 0.42 g/L; Alprazolam: 0.08 mg/L
M	66	PM	Suicide (hanging)	N	Bromazepam: 0.56 mg/L
M	41	PM	Intoxication	Methadone: 823 ng/mL	EtOH: 0.43 g/L
F	ND	IV	Sexual abuse	N	N

EtOH: Ethanol; F: female; IV: *in vivo*; M: male; N: negative results for the studied groups; ND: not determined; and PM: *post-mortem*.

phase extraction and solid phase microextraction and instrumental analysis considers LC–MS, LC–MS(–MS), GC–MS(–MS) and GC–MS.[28] Some show a higher degree of specificity such as a "double equipment" procedure, with detection and quantitation of flunitrazepam and 7-aminoflunitrazepam by HPLC and GC–MS,[29] and thus, not directly compared with the present method results.

Particularly for ketamine detection and quantitation, Brown and Melton have not found a single method using SPE and GC–MS for whole blood samples, confirming the specific difficulties associated with this type of sample.[28] On the other hand, the lack of specific methods in whole blood samples for the same type of chromatographic technique confirms that, as considered above, *post-mortem* samples treatment involves specific issues and obstacles.

The used chromatographic column was also different from the usual published methods, and its smaller length (15 m) allowed smaller retention times, being buprenorphine the most noticeable, since it was possible to achieve a 12.54′ RT, against the usually encountered 20′–25′ RTs in the other methods.

The developed and validated method was then applied to real samples.

From the 14 samples considered, 10 were post-mortem samples and four ante-mortem samples, being these last ones related to suspected sexual abuse cases (Table 5). All the cases had requests for ethanol, illicit drugs, medical substances and the now studied club drugs. The results are shown in Table 5.

It can be noticed the usefulness for ketamine detection, although both cases involved therapeutic concentrations. Methadone was also detected in some cases, with different medico-legal ethiology. It should be noted that the obtained concentrations varied from therapeutic to lethal ones. No other positive results were achieved for the studied compounds, although all the IS and controls used for each sample batch confirmed the method's good performance.

In summary, this paper describes an SPE and GC–MS procedure for quantitative analysis of some club drugs in whole blood samples. The presented method proved to be reliable, with high selectivity, specificity and accuracy for all the studied substances, with very good LOQs, LODs and extraction recovery values. Sample preparation is extremely important to the overall method with respect to increasing the sensitivity and reducing possible interference from the sample matrix. The extraction technique employed allowed good recovery and appropriate selectivity and was, at the same time, simple and reproducible.

The adoption of a SPE procedure with an automatic SPE extraction device, allowed an increased level of automation in sample treatment, being contemporarily less time-consuming, increasing productiveness, always an important matter in forensic laboratories. On the other hand, the procedure itself is easy to use, with a small number of steps, low solvents volume and a buffer use absence. It is also important to state that the simultaneous detection and quantitation of all these compounds by the same extraction and detection methodology is crucial and has a great potential for forensic toxicology and clinical analysis.

Conflict of interest

None declared.

Role of the funding source

There was no external funding applied for or used in writing this article.

Ethical approval

None declared.

References

1. Gahlinger PM. Club drugs: MDMA, gamma-hydroxybutirate (GHB), rohypnol, and ketamine. *Am Fam Physician* 2004;**69**(11):2619–26.
2. Weir W. Raves: a review of the culture, the drugs and the prevention of harm. *Can Med Assoc J* 2000;**162**(13):1843–8.
3. Smith KM, Larive LL, Romanelli F. Club Drugs: methylenedioxymethamphetamine, Flunitrazepam, ketamine hydrochloride, and γ-hydroxybutyrate. *Am J Health Syst Pharm* 2002;**59**:1067–76.
4. Hopfer C, Mendelson B, Van Leeuwen JM, Kelly S, Hooks S. Club drug use among youths in treatment for substance abuse. *Am J Addict* 2006;**15**:94–9.
5. Kelly B. Conceptions of risk in the lives of club drug-using youth. *Subst Use Misuse* 2005;**40**:1443–59.
6. *NIDA info facts. Club drugs*. National Institute on Drug Abuse – National Institute of Health – U.S. Department of Health and Human Services; 2005.
7. Hunt G, Evans K, Wu E, Reyes A. Asian American youth, the dance scene, and club drugs. *J Drug Issues* Fall 2005;**4**:695–732.
8. Simons JS, Gaher RM, Correia CJ, Bush JA. Club drug use among college students. *Addict Behav* 2005;**30**:1619–24.
9. Goldsamt LA, O'Brien J, Clatts MC, McGuire LS. The relationship between club drug use and other drug use: a survey of New York City Middle School students. *Subst Use Misuse* 2005;**40**:1539–55.
10. Britt GC, McCance-Katz EF. A brief overview of the clinical pharmacology of "club drugs". *Subst Use Misuse* 2005;**40**:1189–201.
11. Uys JDK, Niesink RJM. Pharmacological aspects of the combined use of 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) and gamma-hydroxybutyric acid (GHB): a review of the literature. *Drug Alcohol Rev* 2005;**24**:359–68.
12. Brown SD, Rhodes DJ, Pritchard BJ. A validated SPME-GC–MS method for simultaneous quantification of club drugs in human urine. *Forensic Sci Int* 2007;**171**:142–50.
13. Ricaurte GA, McCann UD. Recognition and management of complications of new recreational drugs. *Lancet* 2005;**365**:2137–45.
14. Leshner AI. *Hallucinogens and dissociative drugs. NIDA research report series*. National Institute on Drug Abuse – National Institute of Health – U.S. Department of Health and Human Services; 2001.
15. Thomas Y, Lambert EY. Foreward. *Subst Use Misuse* 2005;**40**:1185–7. Club Drugs Special Issue.

16. Maxwell JC. Party drugs: properties, prevalence, patterns and problems. *Subst Use Misuse* 2005;**40**:1203–40.
17. Musshoff F, Padosch S, Steinborn S, Madea B. Fatal blood and tissue concentrations of more than 200 drugs. *Forensic Sci Int* 2004;**142**:161–210.
18. Stimpfl T, Vycudilik W. Automatic screening in postmortem toxicology. *Forensic Sci Int* 2004;**142**:115–25.
19. Drummer O. Post-mortem toxicology. *Forensic Sci Int* 2007;**165**:199–203.
20. Drummer OH. Postmortem toxicology of drugs of abuse. *Forensic Sci Int* 2004;**142**:101–13.
21. Paterson S, Cordero R, Burlinson S. Screening and semi-quantitative analysis for basic drugs using gas chromatography/ion trap mass spectrometry. *J Chromatogr B* 2004;**813**:323–30.
22. Waters. *Oasis® pharmaceutical applications notebook*. Milford: Waters Corporation; 2002.
23. Waters. *Oasis® applications notebook*. Milford: Waters Corporation; 2002.
24. Castro A. *Validação de métodos internos de ensaio em análise química: Limiares Analíticos do Método de Ensaio – Guide 13*. Lisboa: Relacre; 2000.
25. Christian GD. *Analytical chemistry*. 6th ed. Hobiken: John Wiley & Sons; 2004. Wiley International Edition.
26. Millard BJ, Sheinin EB, Benson WR. Thermal decomposition of propoxyphene during GLC analysis. *J Pharmaceut Sci* 1980;**69/10**:1177–9.
27. The International Association of Forensic Toxicologists. Therapeutic and toxic drug concentrations list, www.tiaft.org [accessed 05.01.11].
28. Brown SD, Melton TC. Trends in bioanalytical methods for the determination and quantification of club drugs: 2000–2010, wileyonlinelibrary.com.
29. Hackett J, Elian AA. Extraction and analysis of flunitrazepam/7-aminoflunitrazepam in blood and urine by LC-PDA and GC–MS using butyl SPE columns. *Forensic Sci Int* 2006;**157**:156–62.